Plant Physiol. (1973) 51, 810–811

Short Communication

Failure of Maleic Hydrazide to Act as a Sulfhydryl or Carbonyl Reagent[1]

Received for publication August 28, 1972

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A wide variety of molecular mechanisms have been proposed to explain the actions of the synthetic plant growth regulator maleic hydrazide (3). One of the most prominent of these theories holds that MH[2] acts as a sulfhydryl reagent (7), and more recently it has been suggested that MH acts as a carbonyl reagent (12). The idea that MH might react with sulfhydryl groups was supported by reports that it can inhibit respiration in several plant tissues and can block several sulfhydryl enzymes (13). Several subsequent studies, however, indicate a lack of effect on both respiration and many sulfhydryl enzymes (1, 3, 4, 10, 13). Furthermore, MH does not decrease the number of thiol groups in radish leaf homogenates (13), and attempts to prevent MH from inhibiting root growth with cysteine were unsuccessful (8). All of these approaches have their limitations, and the question of whether or not MH reacts with thiol groups has not been completely settled. Indeed, it is reasonable that some still accept this mechanism (2). Resolution of this and a related question about reaction of MH with carbonyl compounds is, therefore, essential for understanding MH's action.

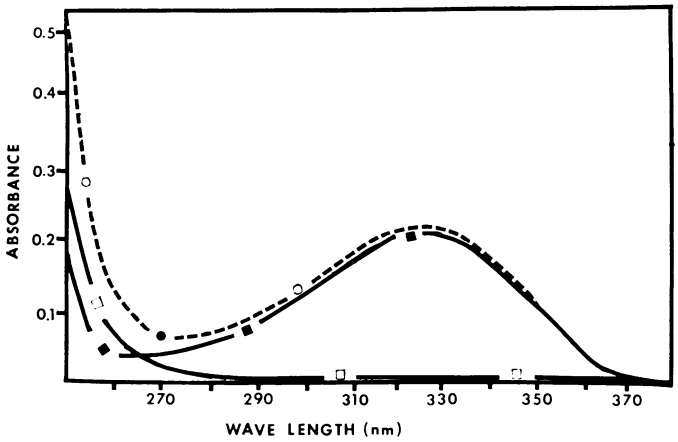

FIG. 1. Comparison of the ultraviolet absorption spectra of 0.1 mM MH (—■—), 5 mM L-cysteine (—□—), a mixture of MH and cysteine at these concentrations (allowed to stand at 25 C for about 30 min) (– –●– –), and the sum of the absorption spectra of MH and cysteine taken separately (– –○– –). All of these solutions and the reference buffer contained 20 mM tris-HCl at pH 7.0. These absorption spectra were measured on a Cary recording spectrophotometer in cells with a 1-cm path length.

Two approaches to these problems are reported here: (*a*) UV spectral studies to determine if MH and sulfhydryl or carbonyl compounds interact in such a way as to cause spectral changes. This would be expected if they were linked covalently, because a double bond would probably be lost or at least the distribution valency electrons would be changed (5, 11). (*b*) Using paper chromatography, a search for possible derivatives formed through reaction with sulfhydryl or carbonyl compounds.

Figure 1 shows the UV absorption spectrum for 0.1 mM MH (recrystallized seven times from hot water) in 20 mM tris, pH 7. Maximal absorption occurs at about 328 nm and minimal at about 265 nm. This corresponds well with spectra reported for MH in sodium pyrophosphate buffer at pH 8.4 (6). Figure 1 also demonstrates that a mixture of freshly made 5 mM cysteine (chosen because it is the thiol suspected to react with MH and it is less hindered stericly than cysteine in protein) and 0.1 mM MH in 20 mM tris, pH 7 (selected to approximate a physiological pH) absorbed UV light to the same extent as the sum of the absorbances of the MH and cysteine separately. Similar results were obtained with 0.2 mM MH and 6 mM cysteine. Thus cysteine and MH do not interact in a way which changes their UV absorption. As expected, a similar mixture of 2 mM cysteine and 1 mM iodoacetate, a well known sulfhydryl reagent, undergoes considerable change in UV absorption; relative to the sum of the individual spectra, the mixture absorbs less from about 249 to about 320 nm and more below 249 nm.

Pyruvate was selected as the carbonyl compound to test for reaction with MH, because it was used to reverse MH inhibition of monoamine oxidase, a result which led to the suggestion that MH acts as a carbonyl reagent (12). When 5 mM pyruvate and 0.2 mM MH are mixed as above in 20 mM tris, pH 7, the mixture again absorbs UV like the sum of the absorbances of the two components taken alone (Fig. 2). As predicted, a mixture of 5 mM $NaHSO_3$ and 1 mM pyruvate does not absorb UV like the sum of the $NaHSO_3$ and pyruvate. Instead UV absorption of the mixture is almost eliminated between 300 and 360 nm, and below 300 nm this mixture absorbs less than the sum of the two components alone. Hence MH does not react with the carbonyl group of pyruvate since this would be expected to change UV absorption of the mixture.

A different type of evidence based on chromatography of MH-1-^{14}C mixed with sodium pyruvate, pyridoxal, or 2-mer-

[1] Supported in part by United States Public Health Service Grant ES-00444.

[2] Abbreviation: MH: maleic hydrazide.

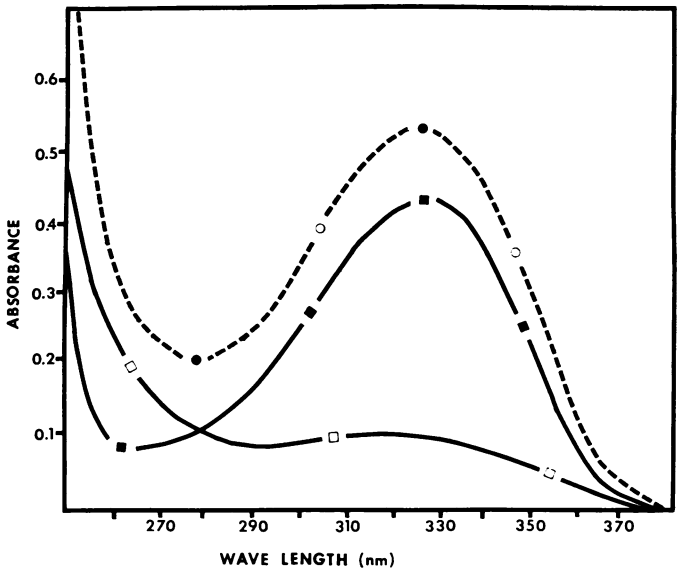

FIG. 2. Comparison of the ultraviolet absorption spectra of 0.2 mM MH (—■—) and 5 mM sodium pyruvate (—□—), a mixture of MH and pyruvate at these concentrations (– –●– –), and the sum of the absorption spectra of MH and pyruvate determined separately (– –○– –). The mixtures were handled and absorption spectra were measured as described for Figure 1.

captoethanol also showed no indication of any reaction product. Sodium pyruvate or pyridoxal in aqueous solutions or mercaptoethanol was mixed with ^{14}C-MH and, after standing 2 hr at 25 C, was chromatographed in the *n*-butanol, acetic acid, H_2O or *i*-propanol, NH_4OH, H_2O or phenol, H_2O solvents described earlier (9). The ^{14}C ran as a single peak, and the R_F was not changed.

Since only a limited number of sulfhydryl and carbonyl compounds were tested, it is hard to be sure that there aren't some other ones which can react with MH; however, those tested should not be unreactive for steric reasons, and they have previously been implicated as reactants.

In view of these results compounded with the previous evidence (1, 3, 4, 8, 10, 13), the theories that MH operates as a sulfhydryl or carbonyl reagent are no longer tenable. The results which supported these theories must have other explanations.

Finally, UV spectral analysis can provide a sensitive measure of changes in the distribution of valency electrons (or even the lack of change) and could be employed more widely in studies on inhibitors and plant growth regulators, especially where formation of a covalent linkage is suspected, and at least one of the reactive groups is a prominent UV chromophore.

Acknowledgment—The author is indebted to Dr. H. W. J. van den Broek for determining the UV absorption spectra.

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